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Mitochondrial membrane potential analysis

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An abbreviated version of this protocol was published in Science Advances in Feb 2021

A combinational chemo-immune therapy using an enzyme-sensitive nanoplatform for dual-drug delivery to specific sites by cascade targeting

DOI: 10.1126/sciadv.aba0776

Detailed protocol

Mitochondrial membrane potential analysis protocol

1. Seed 4T1 cells on coverslips in 12-well plates with at a density of 10^5 cells per well and culture for 24 h.
2. Remove the cell culture medium and wash with PBS two times.
3. Add formulations into each well and incubate for 4h.
4. Remove the supernatant and wash with PBS for two times.
5. Prepare the dyeing working solution mixture containing 0.5 mL culture medium and 0.5 mL JC-1 dyeing solution.
6. Add the dyeing solution to the 4T1 cells and incubate at 37°C for 30 min in the dark.
7. Prepare the JC-1 dyeing buffer solution (1×) and store in ice bath for use.
8. Remove the dyeing solution and wash with JC-1 dyeing buffer solution for two times.
9. Add 1 mL culture medium into wells.
10. Image the stained cells cultured on the coverslips by confocal laser microscope immediately. (E_x/E_m=543/627 nm)

Solutions:

1. JC-1 dyeing working solution: Add 50 μL JC-1(200×) into 8 mL ultrapure water and vortex thoroughly to dissolve JC-1 sufficiently, followed by addition of 2 mL JC-1 dyeing buffer solution inside.
2. JC-1 dyeing buffer solution: Add 1mL JC-1 dyeing buffer solution to 4 mL ultrapure water.
3. The above JC-1 Assay Kits were all purchased from Beyotime Institute of Biotechnology (Jiangsu, China)

Related files

Mitochondrial membrane potential analysis protocol.docx

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cao, J. , Gao, H. and Peppas, N. (2021). Mitochondrial membrane potential analysis. Bio-protocol Preprint. bio-protocol.org/prep1249.
2. He, Y., Lei, L., Cao, J., Yang, X., Cai, S., Tong, F., Huang, D., Mei, H., Luo, K., Gao, H., He, B. and Peppas, N. A.(2021). A combinational chemo-immune therapy using an enzyme-sensitive nanoplatform for dual-drug delivery to specific sites by cascade targeting . Science Advances 7(6). DOI: 10.1126/sciadv.aba0776